CITRIC ACID PRODUCTION BY THE YEASTS *CANDIDA GUILLIERMONDII* AND *YARROWIA LIPOLYTICA*

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ABSTRACT

The aim of this thesis was to investigate the relationships, for a citric acid-producing strain of yeast, among the growth rate, sugar uptake rate and the citric acid production rate, and to investigate the hypothesis that citric acid production occurs when the growth rate slows, but the sugar uptake rate is maintained. As previous experimental work in the Department of Process and Environmental Technology (formerly Biotechnology Department) of Massey University had been performed in shake flask cultures only, it was desired to scale-up the culture into a 2l laboratory scale batch culture, and then into a chemostat culture. The first yeast investigated, *Yarrowia lipolytica* IMK2, failed to successfully scale-up, so further investigations were performed using the yeast *Candida guilliermondii* IMK1.

Experiments were performed in shake flask culture to investigate the effect of using mixed carbon sources to adjust the carbon uptake rate, and hence the citric acid production rate, but no effect was noticed with the mixtures tested.

Batch fermenter experiments were performed to investigate the effect of the culture pH, and the aeration rate, on citric acid production. The aeration rate was not observed to have an effect on the culture in the range tested (0.06 - 0.333 vvm), but the culture pH was observed to have an effect, with the maximum production occurring at pH 4.3, and no citric acid production occurring below pH 3.5.

Chemostat culture experiments were performed to investigate the effect of culture pH and the specific growth rate on citric acid production. The specific

growth rate was observed to have a significant effect, with the specific citric acid production rate increasing as the growth rate decreased. The effect of the culture pH was found to vary with the growth rate, with the maximum production rate and yield occurring at pH 3.8, and a growth rate of 0.02 h^{-1}. From cultures where the glucose was exhausted from the medium, and therefore glucose was a limiting nutrient, the specific citric acid production rate was observed to decrease as the glucose uptake rate decreased. Thus, it could be concluded that the specific citric acid production rate increased as the growth rate decreased, provided that the sugar uptake rate remained high.

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ABBREVIATIONS

ABBREVIATIONS OF UNITS

°C	degrees Celsius
cm	centimetre
g	gram
h	hour
kg	kilogram
kPa	kilopascal
l	litre
M	mole
mg	milligram
min	minute
ml	millilitre
mM	millimole
nm	nanometre
ppm	parts per million
rpm	revolutions per minute
µm	micrometre
v/v	volume per volume
vvm	volume per volume per minute
w/v	weight per volume

OTHER ABBREVIATIONS

AMP Adenosine monophosphate

ATP Adenosine triphosphate

bio biomass (dry weight)

D Dilution rate

DO Dissolved oxygen

EDTA Ethylenediaminetetraacetic acid

HPLC High Performance Liquid Chromatography

N Nitrogen

NAD Nicotinamide Adenine Dinucleotide

q specific growth rate

TCA Tricarboxylic acid

μ Specific growth rate

YNB Yeast nitrogen base

CHAPTER 1

INTRODUCTION

Citric acid is an organic acid produced naturally by most living organisms. Its low toxicity, palatability and ease of assimilation mean that it has many uses, particularly in the food and pharmaceutical industries. It is produced commercially by fermentation of glucose or molasses syrups by strains of the fungus *Aspergillus niger*, or by various yeasts.

Yeast fermentation has some advantages over the fungal fermentation: yeasts are easier to handle in a fermenter as they do not grow on probes or block ports; the form of the growth is usually as a homogenous suspension, rather than in the form of pellets or large aggregates; and they do not require a metal ion deficiency, thus eliminating an expensive medium pre-treatment step. Unfortunately, a side-effect of the yeast fermentation is the occasional by-production of isocitric acid.

Strains of yeast have been developed that can produce citric acid in a nitrogen limited medium containing an appropriate carbon source. The work described in this thesis was undertaken to investigate the relationship between growth rate, sugar uptake rate and citric acid production rate for a strain of yeast grown on glucose, and to test the hypothesis that citric acid production occurs when growth rate slows but the sugar uptake rate is maintained.